Quantitative Sampling of Benthic Macroinvertebrate Communities for Characterizing Response to Mining-Induced Salinization in Appalachia

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Introduction

Decades of coal extraction and streamside residential development in central Appalachia have significantly affected receiving bodies of water, altering stream ecosystem structure and function. Activities associated with surface mining expose un-weathered rocks to water and oxygen, accelerating natural weathering and soluble ion release. Resultant excess leaching of dissolved mineral salts contributes to increased levels of total dissolved solids (TDS) and specific conductance (SC) within receiving headwater streams, culminating in potentially adverse effects on water quality and aquatic life. The negative relationship between increased levels of TDS and freshwater benthic macroinvertebrate community structure is widely documented in headwater streams of the Central Appalachian region (Pond et al. 2008; Timpano et al. 2011, 2015a; Boehme et al. 2016).

Previous studies involving benthic macroinvertebrate community structure in Appalachian streams have relied on U.S. EPA Rapid Bioassessment Protocols (RBP) (Pond et al. 2008; Boehme et al. 2016) and measures such as the Virginia Stream Condition Index (Timpano et al. 2011, 2015a). These studies reported evidence of both seasonal variability in macroinvertebrate community structure and of community structure response to increasing levels of SC, a TDS surrogate, in headwater streams. Methods used in these studies are semi-quantitative and typically involve use of a D-frame net to capture benthic organisms in a small area (~0.3 m^2). This type of sampling, although useful for efficiently estimating benthic community structure through measures of taxon richness, relative abundance, and diversity, does not provide a measure of density of organisms, a metric essential for more accurate characterization of benthic macroinvertebrate community structure. This limitation results from identification of a set number of individual macroinvertebrates (usually 200) regardless of their density (organism #/unit-area of stream substrate) within the collected sample. Furthermore, community structural measures are limited to subsampling of the collected sample, thereby potentially overlooking rare taxa not present in the subsample. This approach limits accuracy of semi-quantitative sampling for characterization of community structure.

In contrast, quantitative sampling relies on complete enumeration of all individuals in a sample, potentially providing more accurate characterization of the benthic community. However, time required to complete sampling is significantly greater for this technique than for semi-quantitative sampling, which relies on a set number of individuals regardless of density. Studies comparing efficiency of different aquatic macroinvertebrate sampling methods have been reported in China (Wang 2006) and Malaysia (Ghani et al. 2016), but no prior studies have conducted quantitative sampling of benthic macroinvertebrate communities across a gradient of

TDS, an environmental stressor, in Virginia's Appalachian region. We expect that quantitative sampling would achieve a more comprehensive and accurate assessment of benthic macroinvertebrate community structure and response to environmental stressors associated with mining in these headwater streams of the Central Appalachian region when compared with semi-quantitative sampling.

Research Goals

The objective of this research was to compare semi-quantitative and quantitative sampling methods for characterizing benthic macroinvertebrate community response to elevated salinity in headwater streams affected by coal mining activity in central Appalachia. The study is addressing the following questions:

1) Do the community metrics measured by the two sampling methods differ?
2) Do community metrics (obtained from each of the two sampling methods) respond to a gradient of SC and TDS?
3) Are the responses comparable between the two methods?

The project is ongoing and this report contains only preliminary results from analysis of data collected in Spring 2014. We initially planned to compare community structure, SC, TDS, and stream chemistry between Spring (April) and Fall (November) seasons within 2014 and within 2017. We also planned to assess changes in community structure, SC, TDS levels, and stream chemistry within Spring and Fall seasons between 2014 and 2017. Due to logistical issues, November 2014 data are not available. Instead, Spring 2014 and 2017 data will be used for comparison as part of this ongoing study.

Methods

Site Selection

Site-selection procedures used for this study are described in Timpano et al. (2015a) and Boehme et al (2016). Fifteen first-order streams were selected from a population of 25 streams that have been assessed for TDS and macroinvertebrate communities using RBP quarterly each year since 2008 (Table 1). 'Reference' streams refer to study sites identified as minimally disturbed by anthropogenic influences, and 'test' streams refer to sites with elevated TDS (Timpano 2011). Rigorous site selection described in Timpano et al. (2015a) ensures that study streams are isolated from non-TDS stressors to the extent possible (Table 2). Specific conductance has also been measured at 15- or -30 minute intervals in each stream since 2011.

Field Methods: Biological Sampling

Each of the 15 streams was quantitatively sampled for benthic macroinvertebrates in April 2014 and May 2017 using a Surber stream-bottom sampler (Surber 1937). Three replicates were collected at each site, for a total of 45 quantitative samples for each collection period. Semi-quantitative benthic macroinvertebrate samples were also collected during April 2014 and May 2017 while following the Virginia Department of Environmental Quality (VDEQ) protocols for single-habitat riffle-run sampling (VDEQ 2008). Three semi-quantitative subsamples were composited for a single sample collected at each stream using a 0.3m-wide D-frame kick-net with 500μm mesh size and preserved in 95% ethanol until laboratory analysis. Sampling in April 2014 was conducted by Damion Drover.

Field Methods: Water-Quality Sampling

Water-quality measurements included long-term, continuous SC and temperature measurements using *in situ* logging meters (Onset HOBO U-24; "dataloggers") installed within the 100m biological survey reach. These automated dataloggers were installed between April and November 2011, and continuously record measurements at 15-minute or 30-minute intervals.

Table 1. Site attributes for selected study streams in the coalfield region of central Appalachia.

Stream	Site ID	Site Type	County	State	Latitude	Longitude	Watershed Area[1] (km^2)
Copperhead Branch	COP	Reference	Dickenson	VA	37.06471	-82.09067	0.81
Crooked Branch	CRO	Reference	Dickenson	VA	37.13013	-82.21794	2.27
Eastland Creek	EAS	Reference	Wise	VA	36.91764	-82.59196	2.38
Fryingpan Creek	FRY	Test	Dickenson	VA	37.06021	-82.21774	5.73
Fryingpan Creek Right Fork	RFF	Test	Dickenson	VA	37.05981	-82.22114	4.56
Grape Branch	GRA	Test	Buchanan	VA	37.25776	-82.00918	4.07
Hurricane Fork	HCN	Reference	McDowell	WV	37.42042	-81.86627	5.93
Kelly Branch	KEL	Test	Wise	VA	36.93472	-82.79085	2.63
Kelly Branch Unnamed Tributary	KUT	Test	Wise	VA	36.93575	-82.79250	1.09
Left Fork/Laurel Fork/Coal Fork	LLC	Test	Kanawha	WV	38.08404	-81.47592	4.17
Mill Branch West Fork	MIL	Test	Wise	VA	36.92717	-82.74680	2.74
Powell River	POW	Test	Wise	VA	37.01310	-82.69751	2.68
Rickey Branch	RIC	Test	Wise	VA	37.03710	-82.54583	4.22
Rockhouse Creek	ROC	Test	Raleigh	WV	37.96569	-81.50123	7.21
Spruce Pine Creek	SPC	Test	Buchanan	VA	37.26124	-81.92038	6.71

[1]determined using data from NHDPlus database (USEPA 2012).

Table 2. Abiotic criteria for selection of reference and test streams (Timpano 2015a).

Parameter of Condition (units or range)	Selection Criterion[1]
Dissolved Oxygen (mg/L)	$\geq$ 6.0
pH	$\geq$ 6.0 and $\leq$ 9.0
Epifaunal Substrate Score (0-20)[2]	$\geq$ 11
Channel Alteration Score (0-20)[2]	$\geq$ 11
Sediment Deposition Score (0-20)[2]	$\geq$ 11
Bank Disruptive Pressure Score (0-20)[2]	$\geq$ 11
Riparian Vegetation Zone Width Score, per bank (0-10)[2]	$\geq$ 6
Total RBP Habitat Score (0-200)[2]	$\geq$ 140
Residential land use immediately upstream	None

[1] Parameters and numeric selection criteria (Burton and Gerritsen 2003).
[2] RBP Habitat, high gradient streams (Barbour et al.1999).

Laboratory Methods: Biological Samples

For April 2014, semi-quantitative biological samples were sub-sampled in the laboratory following RBP (Barbour et al. 1999), and VDEQ Standard Operating Procedures (VDEQ 2006). Biological samples were subsampled randomly to obtain a 200 (±10%) specimen-count. The same procedure will be followed for semi-quantitative samples collected in May 2017. For April 2014, laboratory analysis of quantitative biological samples included macroinvertebrate sorting and identification of all individuals. In each sample, individuals were counted and identified to the lowest practical taxonomic level (usually genus, except individuals in family Chironomidae and sub-class Oligochaeta, which were identified at those levels) using standard keys (Stewart et al. 1993; Wiggins 1996; Smith 2001; Merritt et al. 2008). Taxonomic data were entered into VDEQ's Ecological Data Application System (EDAS) relational database (VDEQ 2010), from which biological metrics were obtained. The same procedure will be followed for quantitative samples collected in May 2017.

Data Analysis

Measurements of SC taken at 15-minute or 30-minute intervals, as well as measurements of TDS at the time of April 2014 macroinvertebrate sampling are completed, downloaded and available for this study. Continuous SC data were summarized to obtain a mean SC value for the year preceding biological sampling dates at each site. Because most benthic macroinvertebrate taxa observed in our study streams require approximately 12 months to mature (Poff et al. 2006) and will therefore be exposed to the full annual range of salinity (Timpano et al. 2015b), mean conductivity levels were quantified for the 12-months prior to April 2014 and were used in data analyses.

Data were analyzed using JMP Pro 12 (SAS Institute, Cary, NC) with test level of $\alpha = 0.05$. Matched-Pair T-tests were performed to detect significant differences in metrics derived from the two sampling methods. Linear regression was used to construct models for April 2014 data, with metric(s) as the response variable(s) and SC as the explanatory or predictor variable. Regression analyses were conducted to determine if responses were comparable between the two methods and to qualitatively determine how the metrics measured by the two sampling methods differ.

Preliminary Results

Total taxa richness derived from the quantitative samples was greater (mean = 35.6) than total taxa richness from the semi-quantitative samples (mean = 20.9) ($p < 0.0001$) (Figure 1). Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa richness derived from the quantitative samples was greater (mean = 23.4) than EPT taxa richness from the semi-quantitative samples (mean = 13.1) ($p < 0.0001$) (Figure 2). The two sampling methods produced measures of relative abundance for EPT (% EPT) and for Ephemeroptera (% Ephemeroptera) that did not differ statistically from one another (Figures 3 and 4).

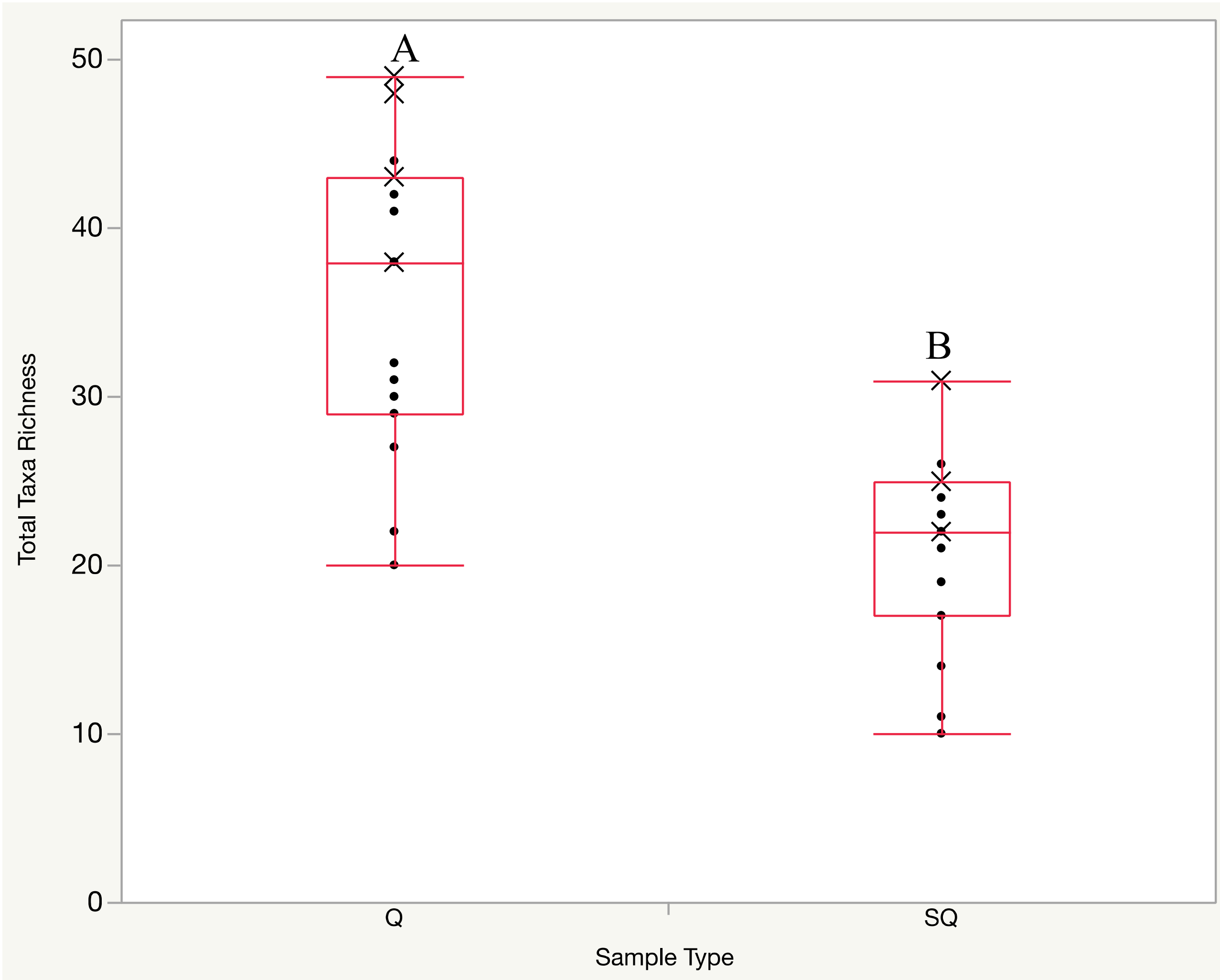

Figure 1. Boxplot of Total Taxa Richness by sampling method (Q = quantitative; SQ = semi-quantitative) for April 2014. n = 15 (4 reference "x", 11 test sites "•"). Different letters indicate significant differences between sample types. Boxes represent the 25th percentile, median, and 75th percentile of measured data; heads and tails represent the 95th and 5th percentiles of measured data.

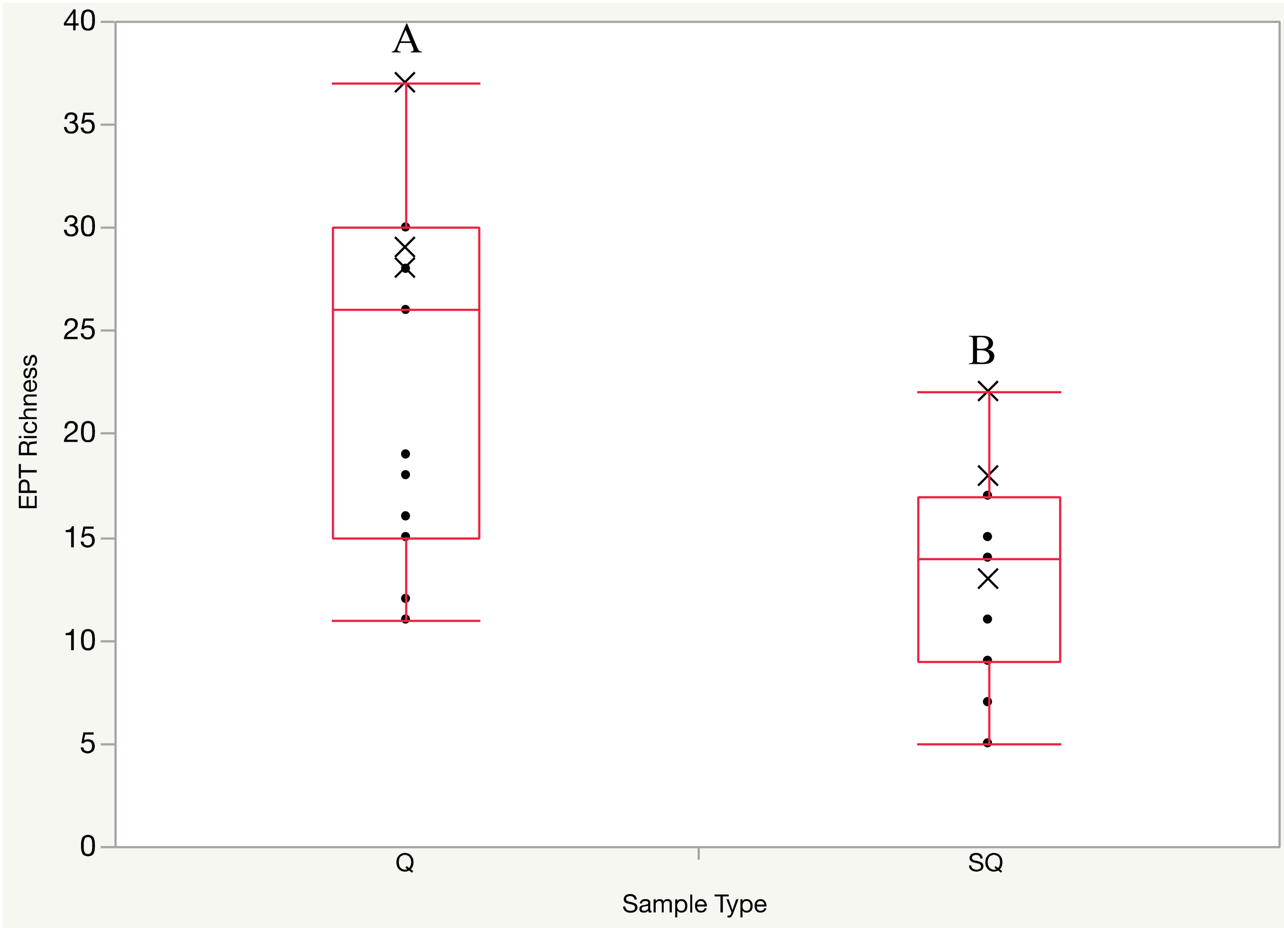

Figure 2. Boxplot of EPT Taxa Richness by sampling method (Q = quantitative; SQ = semi-quantitative) for April 2014. n = 15 (4 reference “x”, 11 test sites “•”). Different letters indicate significant differences between sample types. Boxes represent the 25th percentile, median, and 75th percentile of measured data; heads and tails represent the 95th and 5th percentiles of measured data.

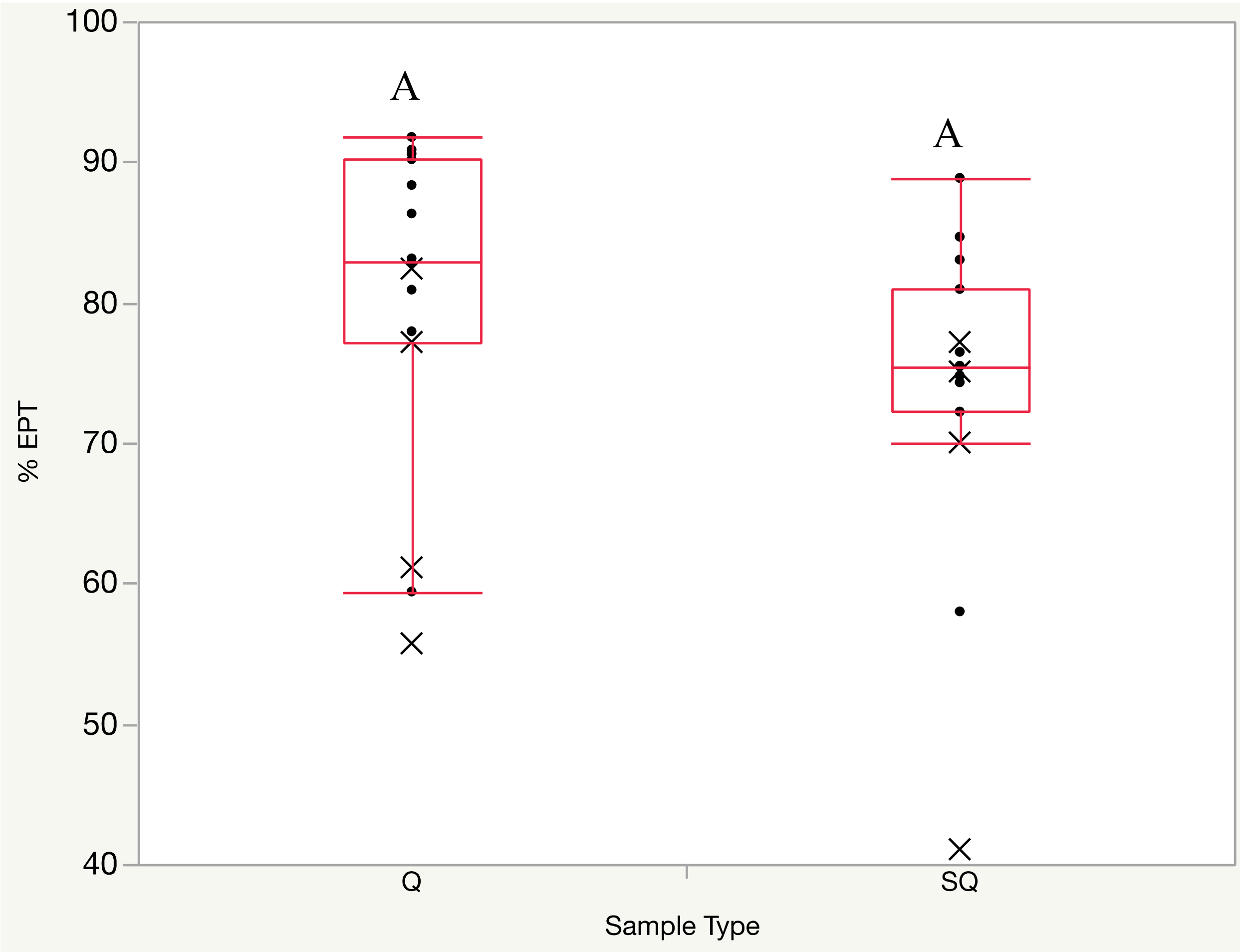

Figure 3. Boxplot of EPT Taxa Abundance (% EPT) by sampling method (Q = quantitative; SQ = semi-quantitative) for April 2014. n = 15 (4 reference "x", 11 test sites "•"). Different letters indicate significant differences between sample types. Boxes represent the 25th percentile, median, and 75th percentile of measured data; heads and tails represent the 95th and 5th percentiles of measured data.

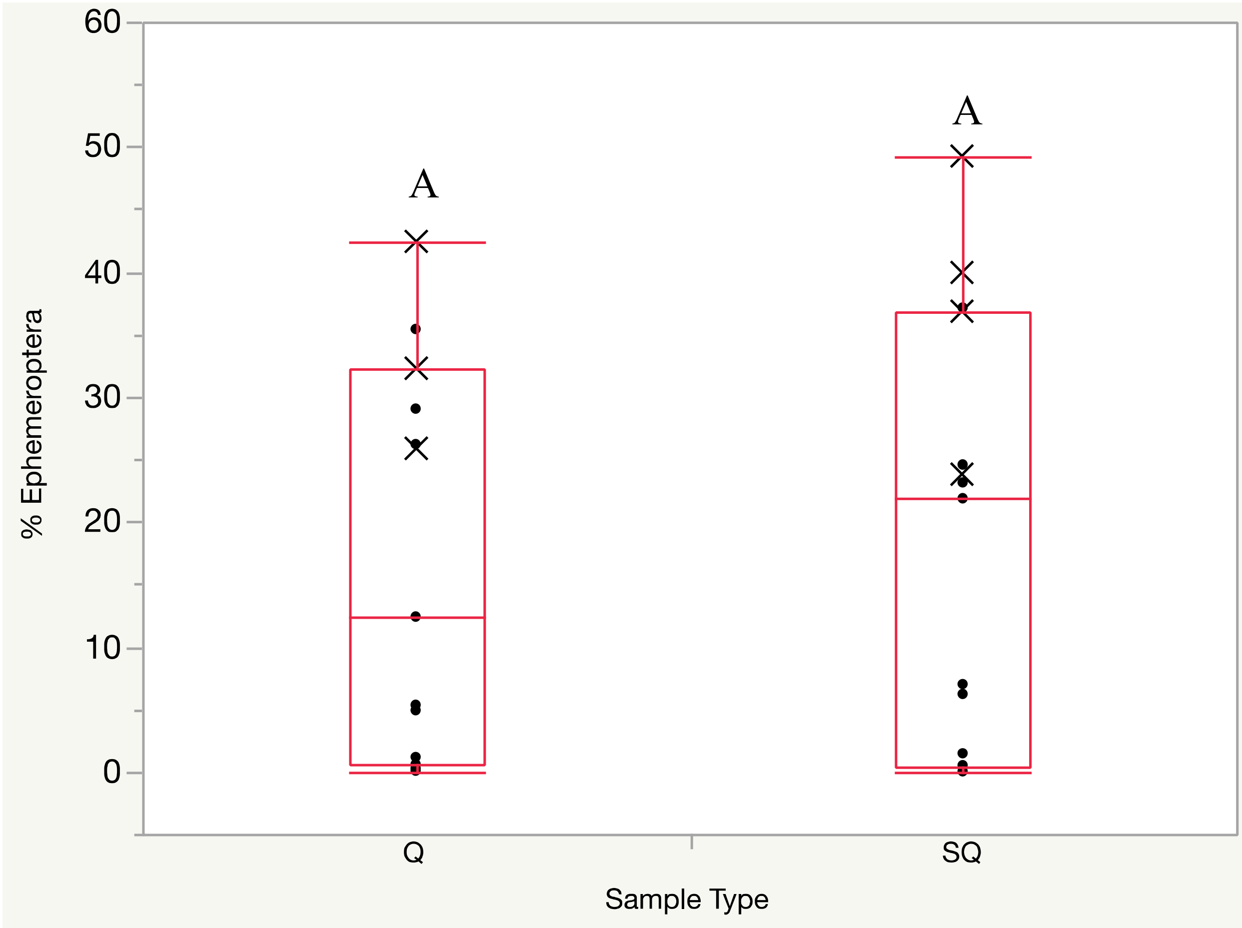

Figure 4. Boxplot of Ephemeroptera Abundance (% Ephemeroptera) by sampling method (Q = quantitative; SQ = semi-quantitative) for April 2014. n = 15 (4 reference "x", 11 test sites "•"). Different letters indicate significant differences between sample types. Boxes represent the 25th percentile, median, and 75th percentile of measured data; heads and tails represent the 95th and 5th percentiles of measured data.

Three of four macroinvertebrate community-structure metrics (total taxa richness, EPT taxa richness, and % Ephemeroptera) exhibited negative relationships with SC for both semi-quantitative and quantitative samples (Figures 5-7), but the % EPT metric did not exhibit a significant relationship with SC for either sampling method (Figure 8).

Linear regression plots for total taxa richness and EPT taxa richness and associated model fits indicate a difference in the SC responses between semi-quantitative and quantitative sampling types for these two metrics, with the slope of the quantitative-sampling response being more negative (Figures 5-7). The SC-regression explains more of the variation in total taxa richness and in EPT taxa richness for the quantitative sampling metrics ($R^2 = 0.66$ and 0.73, respectively) than for the semi-quantitative sampling metrics ($R^2 = 0.29$ and 0.41, respectively) (Figures 5-7). There were no apparent differences in the response slopes for % Ephemeroptera (Figure 8).

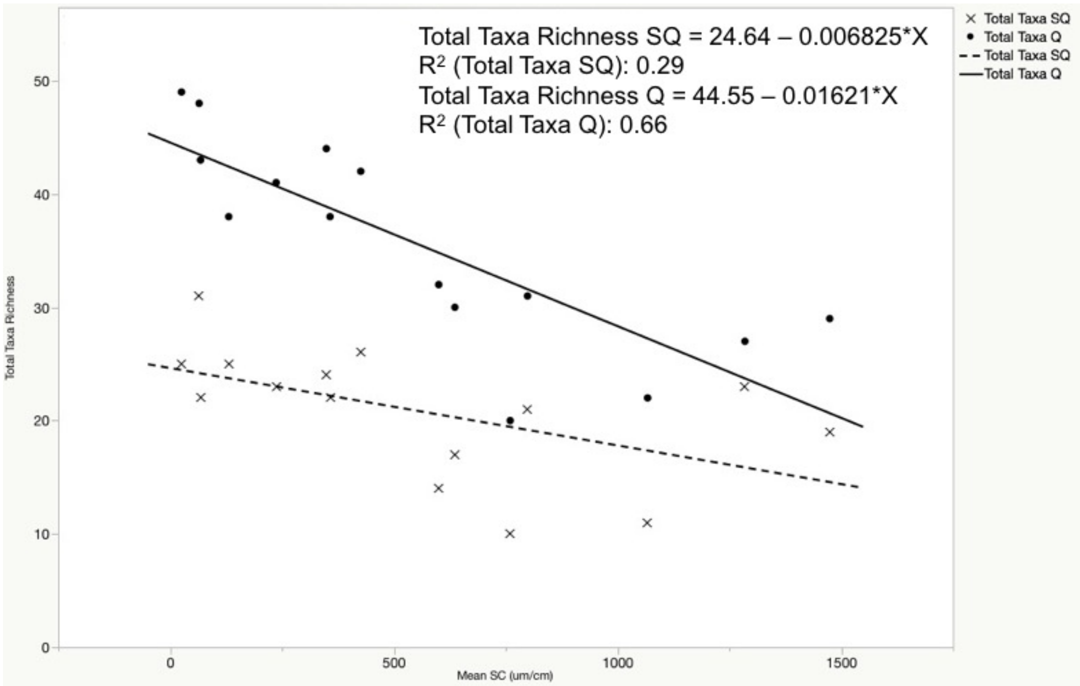

Figure 5. Scatterplot of April 2014 Taxa Richness by mean continuous specific conductance (SC) in 2014. The solid and dotted lines are fitted regression lines, with an associated model fit (adjusted R^2) and equation for the two sampling methods (SQ = semi-quantitative; Q = quantitative).

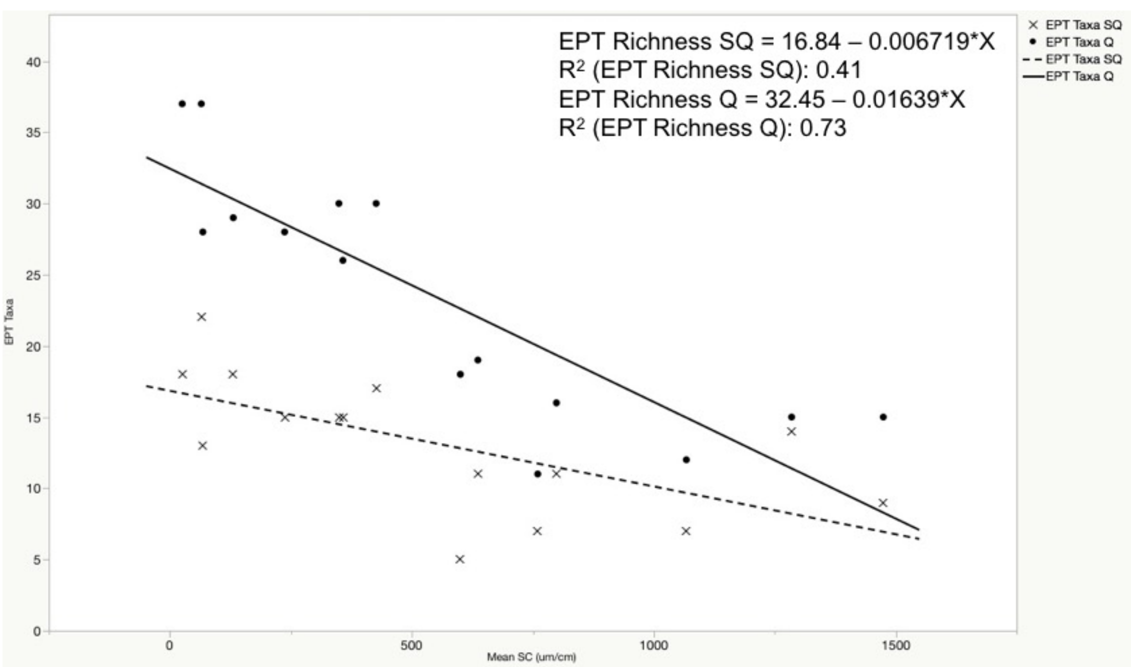

Figure 6. Scatterplot of April 2014 EPT Taxa Richness by mean continuous specific conductance (SC) in 2014. The solid and dotted lines are fitted regression lines, with an associated model fit (adjusted R^2) and equation for the two sampling methods (SQ = semi-quantitative; Q = quantitative).

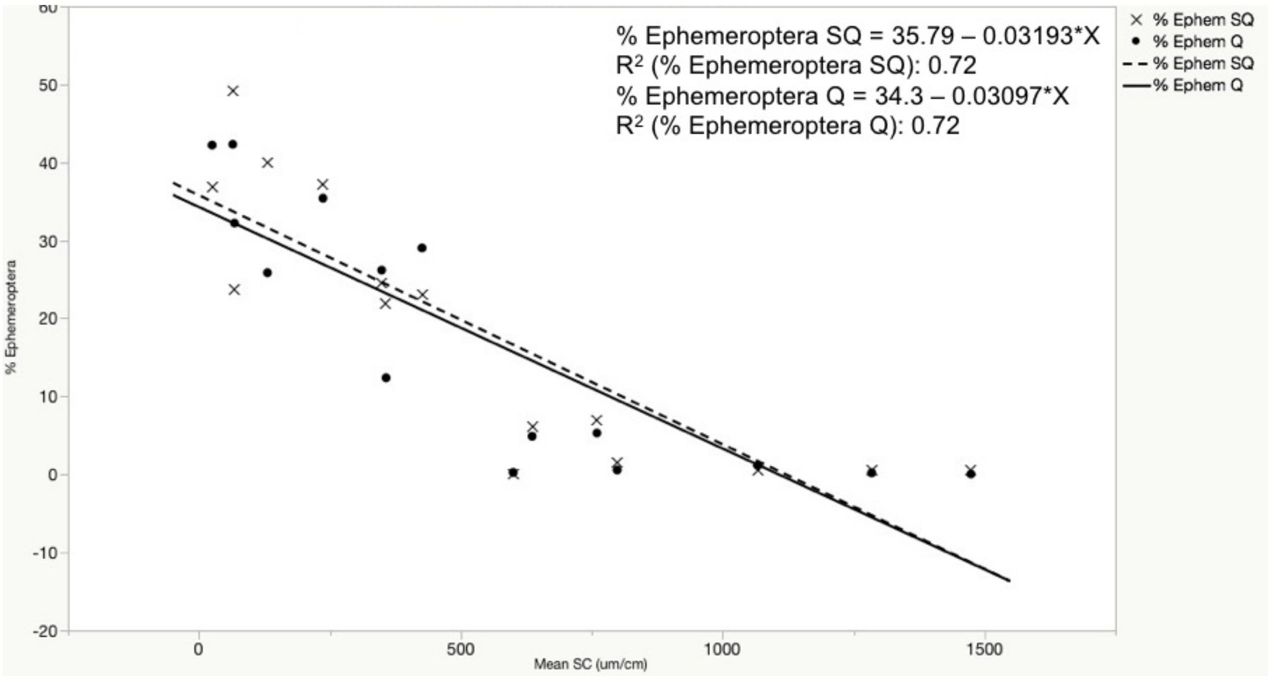

Figure 7. Scatterplot of April 2014 Ephemeroptera Abundance by mean continuous specific conductance (SC) in 2014. The solid and dotted lines are fitted regression lines, with an associated model fit (adjusted R^2) and equation for the two sampling methods (SQ = semi-quantitative; Q = quantitative).

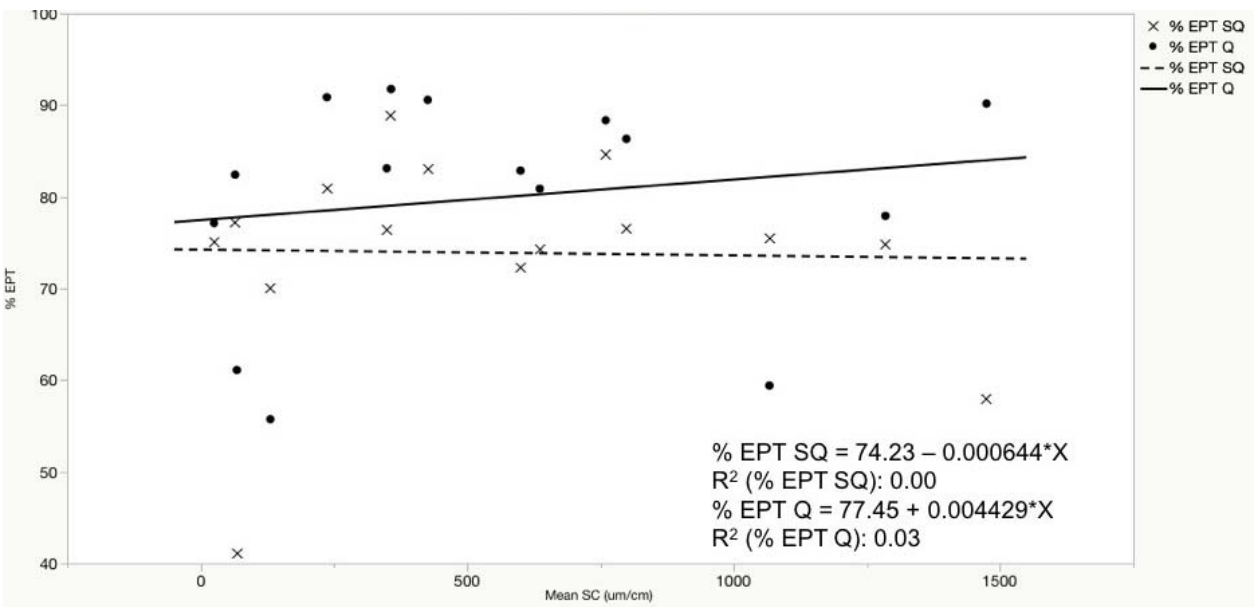

Figure 8. Scatterplot of April 2014 EPT Taxa Abundance by mean continuous specific conductance (SC) in 2014. The solid and dotted lines are fitted regression lines, with an associated model fit (adjusted R^2) and equation for the two sampling methods (SQ = semi-quantitative; Q = quantitative).

Progress Report for Spring 2017 & Future Plans

Additional quantitative and semi-quantitative sampling of benthic macroinvertebrates from each of the 15 study streams was conducted in 2017; however, because of flooded conditions, the sampling period was delayed until mid-late May 2017 ("Spring 2017"). These data are being processed in the laboratory for taxonomic identifications and metric calculations, as are water quality samples collected at the time of benthic macroinvertebrate sampling.

In this report, we evaluated only specific conductance (SC), although other water quality parameters were also measured in Spring 2014 and Spring 2017 and will also be included in the study. In addition to the metrics reported here, several other biological metrics will be calculated and tested for associations with SC and TDS with data from Spring 2014 and Spring 2017 as part of this ongoing study.

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